EXHIBIT 3

BIOalternatives

Study report: GT080450
Proposal: GT080450

Effect of the compound HE immortelle Bio Corse on collagen III production by fibroblasts

L'OCCITANE

Mrs Cécile TOUREL

ZI St MAURICE
BP 307
04100 Manosque
FRANCE

Tel: 04 92 70 25 24

Fax: 04 92 70 19 88

E-mail: ctourel@loccitane.fr

The investigators and the author of this report hereby certify the validity of the data presented and attest their full agreement with the conclusions presented at the end of the report.

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Study director: Franck JUCHAUX, MS
Cellular biology Manager

TABLE OF CONTENT

1. INTRODUCTION

An effect of the compound **HE immortelle Bio Corse** on the collagen III production by dermal fibroblasts was researched.

In this present study, the activity of this compound was evaluated on the stimulation of collagen III synthesis/maturation using an immunofluorescent labelling on human dermal fibroblasts.

ABBREVIATIONS

AU	Arbitrary unit
BSA	Bovine serum albumin
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
FCS	Foetal calf serum
MTT	3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl-tetrazolium bromide)
NHDF	Normal human dermal fibroblast
OD	Optical density
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
RT	Room temperature
Sd	Standard deviation
sem	Standard error of the mean
TGF	Transforming growth factor

2.5. Collagen III production by normal human fibroblasts - immunofluorescence

After incubation, culture media were eliminated. The cells were rinsed with phosphate buffered saline (PBS) solution and fixed with paraformaldehyde (PFA) 4% solution. After saturation of non specific antigenic sites by incubation in PBS-Tween, bovine serum albumin (BSA) 5% buffer, cells were labelled with a primary antibody anti-collagen III (TEBU 600-401-1051) for 1 hour at room temperature.

The primary antibody was then revealed using a fluorescent secondary antibody (GAR-Alexa 488) and the cell nuclei were coloured with Hoechst solution (bis-benzimide).

The acquisition of the images was performed with the INCell Analyzer™ 1000 (GE Healthcare). Controls without primary antibody were performed in order to adjust the acquisition parameters of the camera. Five photos were taken per well. The labelling was quantified by the measurement of the fluorescence intensity (Integration of numerical data with the Developer Toolbox 1.5, GE Healthcare software).

2.6. Data management

The raw data was analysed with Microsoft Excel®.

The inter-group comparisons were performed by Student's t-test. The statistical analysis can be interpreted if $n \geq 5$, however for $n < 5$ the statistical values are for information only.

Formula used in this report:

Standard error of the mean: $sem = Sd/\sqrt{n}$

The standard error of the mean (sem) is a measure of how far the sample mean is likely to be from the true population mean. The sem is calculated as the sd divided by the square root of sample size.

Percentage of Stimulation:

$$\text{Stimulation (\%)} = \left[\frac{\text{Value}}{\text{Mean of control}} \times 100 \right] - 100$$

Percentage of viability: $\%\ \text{viability} = (OD_{\text{sample}} / OD_{\text{control}}) \times 100$

2. MATERIALS AND METHODS

2.1. Biological model

- Cellular type: Normal human dermal fibroblasts (NHDF) pool PF2 used at the 9th passage
- Culture conditions: 37°C, 5% CO_2
- Culture medium: DMEM (Invitrogen 21969035) supplemented with
  Glutamine 2 mM (Invitrogen 25030024)
  Penicillin 50 UI/ml - Streptomycin 50 µg/ml (Invitrogen 15070063)
  Foetal calf serum (FCS) 10% (Invitrogen 10270098)

2.2. Test compound and reference

Test compound	Aspect	Stock solution	Dilution	Test concentrations
HE Immortelle Bio Corse Batch n° OC0611367 Ref. MPBi00iM01 GT080116-3	• Liquid • Storage at RT	1% in DMSO	Culture medium	$8x10^{-5}$, $4x10^{-4}$ and $2x10^{-3}$%

Reference	Stock solution	Dilution	Test concentration
TGF-ß (R&D Systems 240-B-010)	2 µg/ml	Culture medium	10 ng/ml

2.3. Cytotoxicity preliminary assay

- plate format: 96-well
- cells/well: 4000 NHDF in DMEM 10% FCS
- replicates: 3
- concentration range: see Table 1
- cells/compound contact: 72 hours
- evaluation parameter: MTT reduction assay and morphological observations with microscope (objective x10)

2.4. Culture and treatment

The fibroblasts were cultivated in 96-well plates in culture medium. At subconfluence, the medium was removed and replaced by culture medium containing or not (control) the test compound or the reference. The cells were then incubating for 72 hours. All conditions were performed in n=3.

3. RESULTS

3.1. Cytotoxicity preliminary assay

Table 1

The results of the MTT reduction assay and the observation of the cell layers determined, in accordance with the study promoter, the concentrations to be tested (see paragraph 2.2).

3.2. Collagen III production by on normal human fibroblasts

Table 2 and Figure 1

The reference TGF-β significantly stimulated collagen III production by fibroblasts. This result was expected and validated the assay.

The compound **HE immortelle Bio Corse** tested at $8x10^{-5}$% and $4x10^{-4}$% significantly stimulated collagen III production by fibroblasts. At the highest concentration, no effect was observed.

4. CONCLUSION

To conclude, the compound **HE immortelle Bio Corse** show a stimulating effect on collagen III synthesis/maturation by human dermal fibroblasts.

5. TABLES AND FIGURE

Table 1: Effect of the compound **HE immortelle Bio Corse** on the viability of fibroblasts

	Control		HE Immortelle Bio Corse stock solution prepared at 1% in DMSO						Unit %	
			1.3E-07	6.4E-07	3.2E-06	1.6E-05	8.0E-05	4.0E-04	0.002	0.01
Viability (%)	98	101	95	96	102	103	100	97	97	48
	97	107	93	91	98	104	101	103	102	54
	100	97	99	95	98	105	101	107	105	53
Mean	100		95	94	99	104	100	102	101	52
sem	1		2	1	1	0	0	3	2	2
morphological Observations	+		+	+	+	+	+	+	+	-

Legend
+: normal population ; +/-: growth reduction ; -: toxicity ; 0 : cells mortality
g: grains of compound ; op: opacity of the compound ; *: morphological modification ; ag: agglutinated cells
sem : Standard error of the mean (standard deviation divided by sample size square root)

Table 2: Effect of the compound **HE immortelle Bio Corse** on collagen III production by human dermal fibroblasts

Treatment		Basic data					Normalized data		
Treatment	Concentration	Fluorescence Intensity/Number of cells (AU)	Mean (AU)	% Control	sem (%)	$p^{(1)}$	Stimulation (%)	sem (%)	$p^{(1)}$
Control	-	162 168 183	170	100	4	-	0	4	-
TGF-β	10 ng/ml	216 220 210	215	126	2	**	26	2	**
HE Immortelle Bio Corse	$8x10^{-5}$%	192 206 219	206	121	5	*	21	5	*
	$4x10^{-4}$%	187 205 214	202	119	5	*	19	5	*
	$2x10^{-3}$%	195 181 153	176	104	7	ns	4	7	ns

(1): Threshold for statistical significance
ns : > 0.05, Not significant
* : 0.01 to 0.05, Significant
** : 0.001 to 0.01, Very significant
*** : < 0.001 , Extremely significant

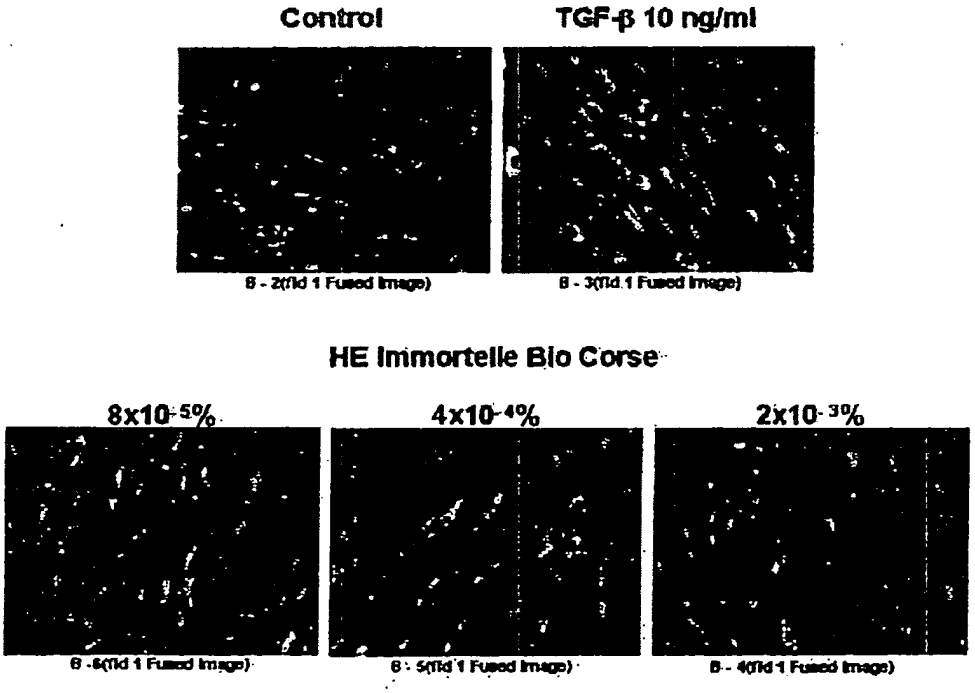

Figure 1: Representative images of collagen III labelling in human fibroblasts